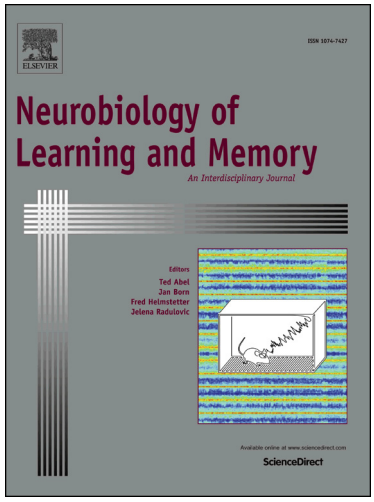

Differential Arc protein expression in dorsal and ventral striatum after moderate and intense inhibitory avoidance training

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Differential Arc protein expression in dorsal and ventral striatum after moderate and intense inhibitory avoidance training

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Abstract

Intense training refers to training mediated by emotionally arousing experiences, such as aversive conditioning motivated by relatively high intensities of foot-shock, which produces a strong memory that is highly resistant to extinction. Intense training protects memory consolidation against the amnestic effects of a wide variety of treatments, administered systemically or directly into brain structures. The mechanisms of this protective effect are unknown. To determine a potential neurobiological correlate of the protective effect of intense training, rats were trained in a one-trial step-through inhibitory avoidance task using different intensities of foot-shock (0.0, 0.5, 1.0, and 2.0 mA). Some rats from each group were sacrificed 45 min after training for immunohistochemical Arc protein detection in dorsal and ventral striatum; other rats were tested for extinction during six consecutive days, starting 48 h after training. The results showed that training with 1.0 and 2.0 mA produced optimal retention scores, which were significantly higher than those of the 0.5 and 0.0 mA groups. Also, a higher resistance to extinction was obtained with 2.0 mA than with the other intensities. A high number of neurons expressed Arc in ventral, but not in dorsal striatum both the 1.0 and 2.0 mA groups, with highest amount of Arc in the neurons of the latter group. We conclude that an increased Arc expression may be related to enhanced synaptic plasticity in the ventral striatum, suggesting that it may be one of the physiological substrates of enhanced learning.

Keywords: striatum, over-training, learning and memory, immediate-early genes, corticosterone

Highlights:

- Intense avoidance training induces Arc expression in ventral striatum.
- Intense avoidance training does not change Arc expression in dorsal striatum
- Arc expression is related to strength of memory but not to foot-shock intensity.

1. Introduction

Information derived from different learning experiences are not similarly stored and retrieved. Compared with events with a neutral connotation, emotional or aversive events are recalled better due to the release of stress hormones, which interact with endogenous neurotransmitters to facilitate memory consolidation (McGaugh, 2013; Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012). Furthermore, stronger consolidation is produced by enhanced learning. Examples of enhanced learning are post-traumatic stress disorder (PTSD) (Parsons & Ressler, 2013), addiction-motivated learning (Hyman, 2005; Torregrossa, Corlett, & Taylor, 2011), and learning produced by intense training (Prado-Alcalá, Medina, López, & Quirarte, 2012). The latter refers to learning mediated by a high number of trials or training sessions, or to one-trial aversive conditioning motivated by high intensities of foot-shock (Prado-Alcalá, 1995; Prado-Alcalá et al., 2007, 2012). The present work focused on intense training.

Intense training produces a strong memory of inhibitory avoidance (IA) which is quite resistant to extinction (Bello-Medina, Flores, Quirarte, McGaugh, & Prado Alcalá, 2016; Garín-Aguilar et al., 2012; Prado-Alcalá, Haiek, Rivas, Roldan-Roldan, & Quirarte, 1994), and to a wide variety of amnestic treatments, administered either systemically or directly into brain structures; these include sodium channel blockers (Garín-Aguilar, Medina, Quirarte, McGaugh, & Prado-Alcalá, 2014; Salado-Castillo, Sánchez-Alavéz, Quirarte, Martínez García, & Prado-Alcalá, 2011), protein synthesis inhibitors (Díaz-Trujillo et al., 2009), and neurotransmitter receptor antagonists (Cobos-Zapiaín et al., 1996; Durán-Arévalo,

Cruz-Morales, & Prado-Alcalá, 1990; Solana-Figueroa, Salado-Castillo, Quirarte, Galindo, & Prado-Alcalá, 2002).

The protective effect of intense training has been studied in several brain structures involved in memory consolidation, such as the hippocampus (Quiroz et al., 2003), amygdala (Salado-Castillo et al., 2011), substantia nigra (Cobos-Zapiaín et al., 1996; Salado-Castillo et al., 2011), and striatum (Pérez-Ruiz & Prado-Alcalá, 1989; Prado-Alcalá & Cobos-Zapiaín, 1977). Importantly, the striatum also has been linked to PTSD (Goodman, Leong, & Packard, 2012), and to the reward-reinforcement learning involved in drug addiction (Yager, Garcia, Wunsch, & Ferguson, 2015).

Growing evidence supports a functional heterogeneity of the striatum. This has led to anatomically dissect the striatum into distinct functional regions or compartments. Taking into account the reciprocal and unidirectional connections with the hippocampus, amygdala, and motor cortical areas, the striatum has been divided into dorsolateral, dorsomedial and ventral regions (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004), albeit other groups suggest a functional distinction between the dorsomedial and dorsolateral regions because the former is predominantly involved in spatial/contextual learning, whereas the latter enables the formation of procedural learning (Devan & White, 1999; Lozano, Serafín, Prado-Alcalá, Roozendaal, & Quirarte, 2013; Packard & Knowlton, 2002; White & McDonald, 2002). We have also reported that there is a differential involvement of its medial and lateral regions in memory consolidation of inhibitory avoidance learning (Salado-Castillo, Díaz del Guante, Alvarado, Quirarte, & Prado-Alcalá, 1996), and that intense training increases dendritic mushroom spines in

dorsomedial, but not in the dorsolateral striatum (Bello-Medina et al., 2016). In addition, cholinergic blockade of dorsal striatum induced retention deficits of an aversively motivated task while blockade of ventral striatum facilitated retention of this task (Neill & Grossman, 1970).

Given the functional heterogeneity of the striatum, the aim of this study was to determine whether different intensities of foot-shock used in inhibitory avoidance training induce distinct patterns of neuronal activation. One strategy to visualize neuronal activation is to measure the expression of immediate-early genes (IEGs). IEG expression is a well-recognized powerful tool for evaluating neuronal activity in the brain; detection of IEG mRNA or protein products in the brain provides information about where and when neurons were activated (Okuno, 2011; Ramírez-Amaya et al., 2005). One such IEG is Arc (Activity-regulated cytoskeletal associated protein, also known as Arg3.1) (Link et al., 1995; Lyford et al., 1995), whose expression is highly dynamic and is induced by robustly patterned synaptic activity, including natural stimuli, seizures, LTP, and memory-related processes (Guzowski, 2002; Guzowski et al., 2000; Guzowski, Setlow, Wagner, & McGaugh, 2001; Lyford et al., 1995; Ramírez-Amaya et al., 2005).

Exploration of new environments induces strong Arc expression in the hippocampus as well as in related neocortical areas such as the parietal and entorhinal cortex in rats and mice (Guzowski, McNaughton, Barnes, & Worley, 1999; Ramírez-Amaya et al., 2005). Arc involvement in learning and memory processes is further supported by the deterioration of long-term potentiation and spatial water maze learning produced by inhibition of its expression with infusion of antisense oligodeoxynucleotides directly into the hippocampus (Guzowski et al.,

2000); this inhibition also impairs long-term, but not short-term memory of inhibitory avoidance (McIntyre et al., 2005). Furthermore, it was found that in the hippocampus and cortex of rats the proportion of cells expressing Arc protein is the same as that of cells that express Arc mRNA after spatial exploration (Ramírez-Amaya et al., 2005).

Although Arc mRNA expression in dorsomedial or dorsolateral striatum alone did not correlate with the stage of training in a dual-solution task (a task that can be solved using either place or response strategies), the ratio of expression in the dorsomedial striatum to that in the dorsolateral striatum was relatively high among rats that used a place strategy early in training, as compared with the ratio among over-trained response rats (Gardner et al., 2016). Together, these data stress the importance of Arc protein in the consolidation of different types of memory, as well as its usefulness for mapping neuronal networks that underlie information processing.

In sum, the studies described above showed that there is a regional differentiation within the striatum regarding the processing of information involved in memory of diverse tasks, mediated by moderate or intense training. In the present work we hypothesized that moderate training of inhibitory avoidance induces the expression of Arc protein in different striatal regions; we also predicted that intense training will induce a higher expression of this protein in different striatal regions.

In order to test these hypotheses, we evaluated the expression patterns of the IEG Arc in the dorsomedial, dorsolateral, ventromedial, and ventrolateral regions of the striatum after training rats in the inhibitory avoidance task with

different foot-shock intensities. Additionally, as an indication of the extent of emotional arousal produced by the task, we quantified blood serum corticosterone (CORT) concentration, which correlates positively with the intensity of aversive training (Cordero, Merino, & Sandi, 1998).

2. General methods

This section describes the procedures common to the different experiments of this study.

All experimental procedures were approved by the Animal Ethics Committee of the Instituto de Neurobiología, Universidad Nacional Autónoma de México, which complies with the Mexican Council Norm (SAGARPA NOM-062-ZOO-1999) and the NIH Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.1 Subjects

We studied 98 adult male Wistar rats (250–350 g; 8-9 weeks old on arrival to the laboratory vivarium), which were maintained in a room with a 12/12 h light-dark cycle (lights on at 7:00 h) and housed individually in acrylic cages with food and tap water ad libitum. The temperature of the room where the animals were housed was kept at 21 °C.

2.2 Handling

Rats were handled, once a day, for 5 min across 3 days prior to the experiments. The handling procedure consisted of gently touching and holding the

rat with both hands. All animals were handled and transported from the vivarium to the handling room, including a group that never experienced the inhibitory avoidance task nor the training room (group Cage). All the experiments were performed between 9:00 h and 13:00 h.

2.3 Apparatus

The rats were trained in an inhibitory avoidance apparatus consisting of two compartments separated by a sliding door. The safe compartment (30 x 30 x 30 cm) had a lid and walls made of red-colored acrylic, with a floor made of stainless steel bars (6 mm in diameter, 9 mm apart). This compartment was illuminated by a 10 Watt light bulb located in the center of its lid. The other, non-illuminated shock compartment (30 cm long) had front and back walls and a floor made of stainless steel plates with side walls and lid constructed of red-colored acrylic. The walls and floor were shaped like a trough, 20 cm wide at the top and 8 cm wide at the bottom. In the middle of the floor, a 1.5-cm slot separated the two stainless steel plates that make up the walls and floor. Upon entering the non-illuminated compartment, the rats were in contact with both plates through which a foot-shock could be delivered. A square-pulse stimulator (Grass model S-48) in series with a constant current unit (Grass model CCU-1) generated the foot-shock. Shock delivery and measurement of latencies to cross from one compartment to the other one were accomplished by use of automated equipment. Both compartments were wiped with 10% alcohol before and after each trial. The apparatus was located inside a dark, sound-proof room provided with background masking noise.

2.4 Statistical analysis

Because the measurement of retention of the IA task was truncated at 600 s, nonparametric statistics were used in analyzing the behavioral results. Independent Kruskal–Wallis ANOVAs were computed for acquisition and escape latencies. When appropriate, the Mann–Whitney U test was used to make comparisons between any two groups. In the experiment on extinction the Friedman test was used to analyze retention latencies across the six days of testing for each group, followed by the Wilcoxon Signed-Rank Test to make comparisons between any two days along the extinction sessions in each group. To compare retention scores among the groups on each day of extinction testing the Kruskal-Wallis and the Mann-Whitney U tests were applied. For the histological results, independent one-way ANOVAs were used to analyze the area of Arc signal, the percentage of Arc-expressing cells, as well as the CORT serum concentration. When the F-ratios were significant, a Bonferroni multiple comparisons test was used to carry out pairwise comparisons.

3. High foot-shock intensity during the inhibitory avoidance training produces enhanced learning

Because one of the main objectives of this study was to evaluate possible changes in the expression of Arc after low, moderate, and intense training, it was important to demonstrate that training with 2.0 mA produces stronger learning than training with 0.0, 0.5, or 1.0 mA. To this end we measured resistance to extinction, which is stronger when a learning experience is also stronger.

On the day of training, each rat was placed inside the safe compartment and 10 s later the door between the two compartments was opened. The latency to cross from the safe to the shock compartment is referred to as the training latency. Once the animals crossed to this compartment the door was closed and a foot-shock was delivered (a train of 50 ms square pulses at 10 Hz, with intensities of 0.5, 1.0, or 2.0 mA). Five seconds later the door was reopened, allowing the rat to escape to the safe compartment and then the stimulator was turned off; this latency is referred to as the escape latency. After 30 s in the safe compartment the rat was placed back in its home cage. The 0.0 mA group was trained in the inhibitory avoidance task, except that the foot-shock was not delivered. In this group, once the rats crossed into the shock compartment, the door was re-opened after 5 s, and the latency to return to the safe compartment was computed; there were 10 rats per group.

Extinction of the task was measured on six consecutive days, starting 48 h after training. The same procedure as in training was followed, except that the foot-shock was omitted; if the rat did not cross into to the shock compartment within 600 s, the session ended and a retention latency score of 600 s was assigned.

3.1 Results

The Kruskal-Wallis test demonstrated that there were no significant differences in the training latency among the groups ($H(3) = 5.06$, $p = 0.17$) (Figure 1A). In the case of escape latencies significant differences were observed among the groups ($H(3) = 13.90$, $p < 0.005$). Pairwise comparisons revealed that the 0.0 mA group had a significantly higher escape latency compared to the 0.5, 1.0, and

2.0 mA groups (p´s < 0.01, 0.005, and 0.05, respectively). The escape latencies of the latter three groups did not differ from each other (Figure 1B).

As depicted in Fig. 1C, the Friedman test revealed significant differences throughout the extinction sessions in the 0.5 ($X^2(5) = 26.36$, $p < 0.001$); 1.0 ($X^2(5) = 37.42$, $p < 0.001$); and 2.0 ($X^2(5) = 29.89$, $p < 0.001$) mA groups, while no differences were found in the 0.0 mA group ($X^2(5) = 6.38$, $p = 0.23$). The Wilcoxon matched-pairs test showed that the 0.5 mA group had a significant reduction in the retention latency on the third extinction day compared to the first extinction day ($Z = 36$, $p < 0.01$) and continued low during the remaining sessions ($Z = 36$, $p < 0.01$ for the fourth, fifth, and sixth extinction days). The 1.0 mA group had a significant decrease in the retention latency on the second extinction day compared to the first day ($Z = 26$, $p < 0.05$). This reduction was more evident on the third extinction day and remained low up to the sixth day ($Z = 55$, $p < 0.005$ from the third to the sixth extinction day). The 2.0 mA group had the maximum median retention latency (600 s) during the first three extinction sessions; on the fourth, fifth and sixth days, a significant reduction on the retention latencies was observed ($Z = 21$, $p < 0.05$ for both fourth and fifth days; $Z = 36$, $p < 0.01$ for sixth day).

Session by session comparisons of retention scores among the groups across the six extinction sessions yielded significant differences ($H(3)$ = range 17.34 to 35.25, $p < 0.001$ in each session). As depicted in Fig. 1C, group 2.0 mA had higher retention scores on every session than the rest of the groups (p values ranging between 0.001 and 0.0001), except for the first session where it did not differ from the 1.0 mA group. In turn, the 1.0 mA group had significantly higher scores than the 0.5 mA group only on the first session ($p < 0.005$), while it differed

from the 0.0 mA group on the first five sessions (*p* values ranging from 0.01 to 0.001) but not in the sixth session. Lastly, the 0.5 mA group had higher retention latencies than the 0.0 mA group only in the first and third sessions (*p´s* < 0.001 and 0.05, respectively).

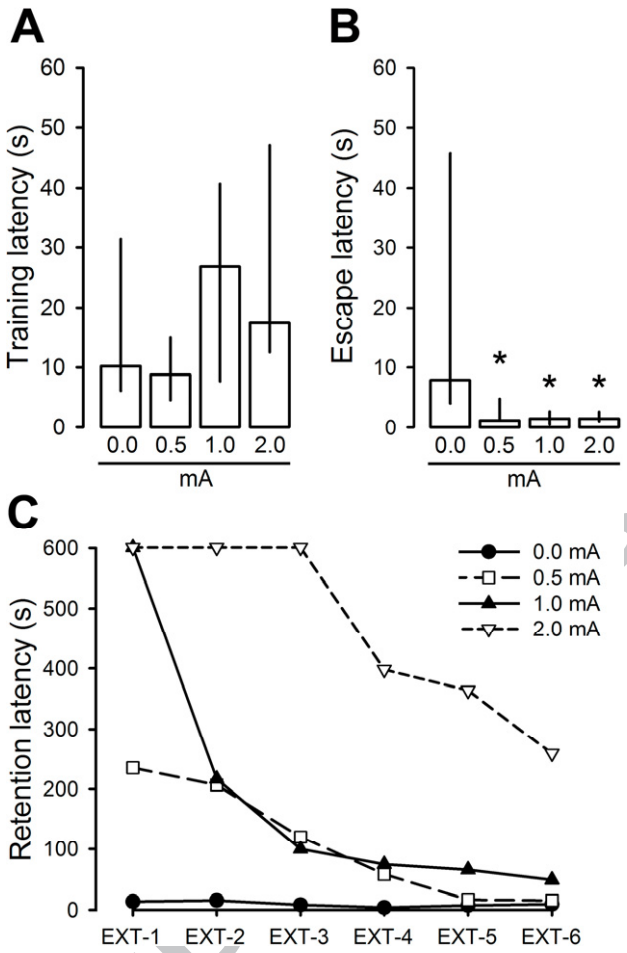

Figure 1. Performance of groups of rats trained in inhibitory avoidance with 0.0, 0.5, 1.0, or 2.0 mA. (A) Median training latencies (with interquartile ranges). (B) Median escape latencies (with interquartile ranges). (C) Median retention latencies across the daily extinction sessions (EXT-1 to EXT-6). In every extinction session retention scores of the 2.0 mA group differed significantly from the latency scores of the other groups (*p* values ranging between 0.001 and 0.0001), except on EXT-1 where it did not differ from the 1.0 mA group (n = 10 per group). These results clearly indicate that the 2.0 mA group had the highest resistance to extinction, which is indicative of enhanced learning. * $p < 0.05$ vs. 0.0 mA.

4. Intense inhibitory avoidance training increases Arc protein expression in ventral but not in dorsal regions of the striatum

In order to explore the effects of moderate and intense training on neuronal activation of the striatum, groups of rats were trained using 0.0, 0.5, 1.0, or 2.0 mA; none of these groups was tested for retention of the task, and they were sacrificed 45 min after training. The 0.0 mA group was trained in the inhibitory avoidance task, except that the foot-shock was not delivered. In this group, once the rats crossed into the shock compartment, the door was re-opened after 5 s, and the latency to return to the safe compartment was computed. We also studied another group to factor out the effects of the foot-shock. This group received the highest intensity foot-shock (2.0 mA), but was not trained in the avoidance task (shock-only group; SO-2); the rats of this group were placed directly into the shock compartment and a 2.0 mA foot-shock was delivered (the foot-shock duration was 6.5 s, which was the median of the duration of the foot-shock received by the 2.0 mA trained group). Immediately after the foot-shock, the rat was placed back in its home cage. In order to determine the basal expression of Arc protein we also studied a caged control group that never experienced the inhibitory avoidance task nor the training room (group Cage). There were 4 rats in each group, and these same rats were used to measure serum concentration of CORT (see below).

4.1 Brain extraction and cryosectioning

Forty-five min after the manipulations just described the rats were decapitated and their brains were removed and immediately frozen in 2-methylbutane (Sigma-Aldrich) immersed in a slurry of dry ice and ethanol. After

freezing, the brains were stored at –70 °C until cryosectioning. Using a stainless steel matrix (Electron Microscopy Sciences), brain hemi-sections that included the whole striatum were obtained. The hemi-sections were placed in a mold and a block was made with Tissue-Tek OCT compound (Sakura Finetek), such that each block contained eight brains, at least one from each group, and their position differed in each block. Each of the resulting three blocks were coronally cryosectioned at –20 °C to a thickness of 20 µm, using a CM1850 Leica cryostat. The brain slices were placed on slides and kept in a sealed box at -70 °C prior to the immunostaining procedure.

4.2 Immunohistochemistry

Six slides from each block from the striatum (Bregma 0.24 to 1.20; Paxinos & Watson, 2007), were randomly selected, and the tissue was fixed in 2% paraformaldehyde, 7.4 pH, for 5 min, and washed in 2× SSC, pH 7.0, followed by 50:50% acetone/methanol at 4 °C for 5 min. The tissue was washed in 2× SSC and 0.05% Tween 20 and quenched in 2× SSC and 1% H_2O_2 for 15 min. After blocking with a tyramide signal amplification kit (TSA) blocking buffer (PerkinElmer Life Sciences), the slides were incubated in polyclonal rabbit anti-Arc antibody (1:800) (kindly supplied by Dr. Hiroyuki Okuno) for 48 h at 4 °C. Incubation with the anti-rabbit biotinylated secondary antibody (Vector Laboratories) for 2 h at room temperature was followed by amplification with the avidin-biotin system (Vector Laboratories) for 45 min. The immunostaining was visualized using the cyanine 3 (CY3) TSA fluorescence system (PerkinElmer Life Sciences), and the nuclei were counterstained with SYTOX® Green (Molecular Probes). No immunostaining was

detected in the absence of the primary or secondary antibodies. Immunostaining for Arc usually covered between 17 and 19 μm of the thickness of the tissue; the vast majority had 18 μm, and all slides with staining covering less than 17 μm were excluded from analysis.

4.3 Image acquisition and analysis

Single optical plain images covering the 20 μm thickness of the tissue were obtained with an Apotome system (Zeiss), using a 10x/0.45 apochromatic objective lens and a filter set for CY3 detection with a bandpass excitation of 546/12 nm and emission of 575-640 nm, and a filter set for SYTOX® Green detection with a bandpass excitation of 450-490 nm and emission of 515-565 nm. With the aid of the MosaiX module these images were used to perform image stitching to build six mosaic images per animal. Each mosaic image was obtained in order to cover the whole striatum (Figure 3). The parameters for the image acquisition were set in the slice of the animal from the Cage group in each slide. Once these parameters were set, they remained constant for the rest of the brain slices in that slide.

Analysis of the Arc expression was performed on the images using the ImageJ software (Schneider, Rasband, & Eliceiri, 2012). Because the number of images that would be used to count the total number of neurons was very high (1,728 images), we decided to first do an area analysis approach, which allowed us to detect the striatal regions that showed changes in the expression of Arc, and then to do the cell count on the slides of such regions.

For this type of analysis, we divided the anterior striatum into four regions: dorsomedial (DM), dorsolateral (DL), ventromedial (VM) and ventrolateral (VL)

(Figure 2). This division was based on mapping studies of the striatum (Prado-Alcalá & Wise, 1984; Salado-Castillo et al., 1996). The boundary between the anterior and posterior regions was defined as the coronal plane 0.2 mm posterior to bregma. At each anterior-posterior level the medial-lateral dividing line was taken as half the distance between the most medial and most lateral extent of the striatum for that section; similarly, the dorsal-ventral dividing line was halfway between the most dorsal and the most ventral extent of the striatum for a given section. Then a detection threshold on the grayscale value (defined as the grayscale value that was at least twice as large as the background noise), was established on a tissue slice from an animal of the Cage group in each analyzed slide; this threshold value was used for the detection of Arc signal area of all the tissue slices of the same slide. This threshold-setting was used as a stereological measure to control the variation in the signal produced by the immunostaining process. Lastly, the area of Arc signal that reached the threshold was measured in each delimited region (expressed in μm). In order to minimize the variations due to the histological procedures, such as shrinkage or flattening of the tissue, we divided the Arc signal area by the total area of each given striatal region (also expressed in μm), thus obtaining the Arc signal area ratio.

Subsequently, in those striatal regions where significant differences in Arc signal area ratio were found, three sample z-stack images, from the same slices previously analyzed, were obtained for cell counts (0.7 μm optical thickness per plane, approximately 26 ± 2 planes of optimal staining per image, 40× objective) (Fig. 2). The cell count method was previously reported by Vazdarjanova, McNaughton, Barnes, Worley, & Guzowski, (2002). Briefly, this method consists of

identifying and classifying neurons on the basis of the nuclear counterstain, thereby discriminating neurons from glial cells; glial nuclei are small, with intense and uniform DNA staining, while neuronal nuclei are larger and have a less intense and non-uniform DNA staining. Only the neuron-like cells found in the middle 60% of each stack were included in the analyses (this stereological measure was used to minimize sampling errors attributable to incomplete cells). The probability of a biased cell count analysis regarding different cell populations was negligible because the vast majority of striatal neurons (95-98%) are medium spiny neurons, which have an approximate diameter of 12-14 microns (Dudman & Gerfen, 2015; Kemp & Powell, 1971; Kreitzer & Malenka, 2008). For Arc protein analysis, the selected cells were classified as either positive or negative. Positive neurons had perinuclear or cytoplasmic staining surrounding at least 60% of nucleus circumference, and had to be visible in at least three planes together with the cell nucleus. Special attention was paid to ensure that the staining belonged to the cell of interest and not to a dendrite of a different cell.

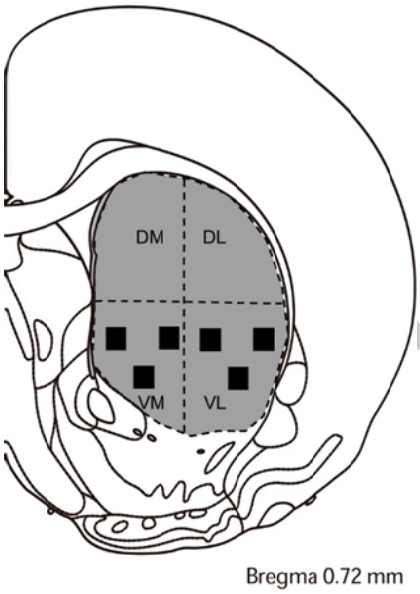

Figure 2. Schematic diagram illustrating the location of the four striatal regions used for the signal area measurement (gray areas), and the areas where the cell count (black squares) was performed. Abbreviations are as follows: DM, dorsomedial; DL, dorsolateral; VM, ventromedial; VL, ventrolateral. Drawing modified from Paxinos & Watson (2007).

4.4 Results

4.4.1 Signal area analysis

The signal area measurements of Arc protein obtained from mosaic images revealed no significant effects of treatments in DM and DL regions of the striatum ($F(5, 18) = 1.69$, $p = 0.19$; and $F(5, 18) = 2.15$, $p = 0.11$, respectively) (Figure 3A and 3B). In contrast, the ventromedial striatal region showed significant differences among the groups ($F(5,18) = 3.9$; $p < 0.05$), and the Bonferroni post hoc test revealed significant differences in Arc signal area between the 2.0 mA and the SO-2 groups ($p < 0.05$); there were no significant differences when pairwise comparisons were made with the rest of the groups (Fig. 3 C).

In the ventrolateral region of the striatum significant differences were also found among groups ($F_{(5,18)} = 3.76$; $p < 0.05$). The Bonferroni post hoc analysis revealed that Arc signal area was significantly higher in the 2.0 mA group than in the Cage, 0.0 mA, and SO-2 groups (p´s < 0.05, 0.05, and < 0.01, respectively). Bonferroni pairwise comparisons showed that the rest of the groups did not differ significantly from each other (Figure 3D).

As stated above, the signal area measurement revealed that no changes in Arc protein expression in DM and DL striatum were produced by any of the experimental manipulations. By contrast, in the ventral striatal regions training with the higher foot-shock (2.0 mA) induced Arc protein expression particularly in the ventrolateral striatal regions. This increase in Arc protein could be related to a higher concentration of Arc protein in the active neurons, to a higher number of active neurons, or both. In order to determine the contribution of the number of

active neurons to this outcome, a cell count was performed in the ventromedial and ventrolateral regions of the striatum.

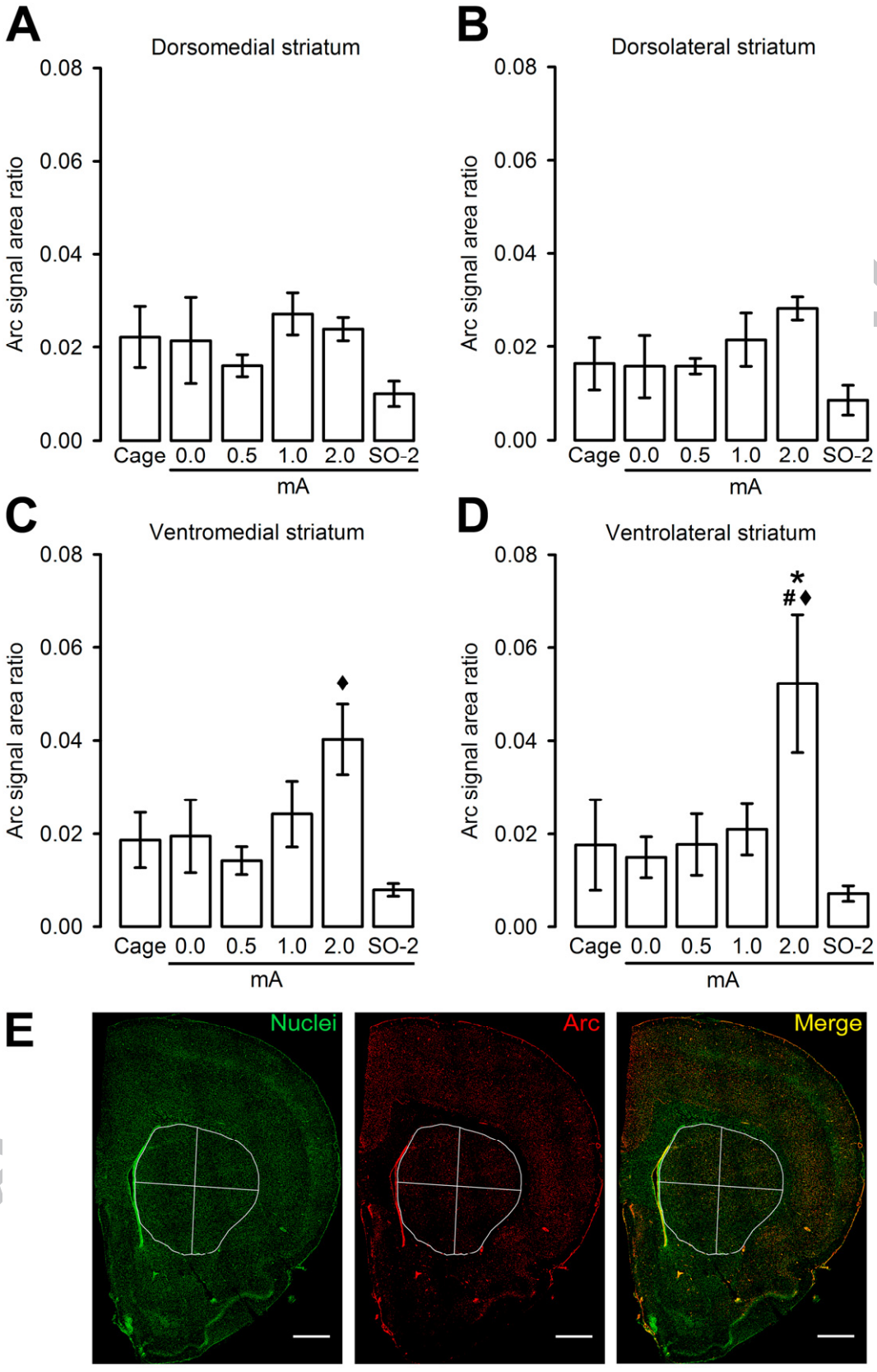
A
Dorsomedial striatum
B
Dorsolateral striatum
C
Ventromedial striatum
D
Ventrolateral striatum
Arc signal area ratio
0.08
0.06
0.04
0.02
0.00
Cage
0.0
0.5
1.0
2.0
SO-2
mA
*
#
♦
E
Nuclei
Arc
Merge

Figure 3. Effects of intensity of inhibitory avoidance training on Arc signal area in four different regions of the striatum. There were no significant differences in *Arc* signal *(mean ± SEM),* among the groups in dorsomedial (A) and dorsolateral (B) striatum. A higher mean area of Arc signal was found in the groups trained with the high foot-shock intensity as compared to the SO-2 group in ventromedial (C) and ventrolateral (D) striatum (n = 4 per group). *$p < 0.05$ as compared with the Cage group, ♦$p < 0.05$ as compared with the SO-2 group, # $p < 0.05$ as compared with the 0.0 mA group. (E) Representative images of SYTOX-green counterstain for nuclei detection (green), Arc detection (red), and a merge image of Arc and nuclei from an animal trained with 2.0 mA. Scale bar = 500 µm.

4.4.2 Cell count

The one-way ANOVA showed significant differences among the groups in the proportion of Arc expressing neurons of the ventromedial striatal region ($F_{(5,18)}$ = 11.76, $p < 0.0001$). The post hoc Bonferroni test revealed a higher proportion of Arc-expressing neurons in the 2.0 mA group as compared to the Cage, 0.0, 0.5 mA, and SO-2 groups ($p´s < 0.001$, 0.005, 0.005, and 0.001, respectively). Group 1.0 mA also had a higher proportion of Arc-expressing neurons than the Cage and SO-2 groups ($p´s < 0.01$ and 0.005, respectively), but it was not significantly different from the others groups; particularly important is the lack of significance with the 0.0 and 0.5 mA groups, indicating that the increase in the number of Arc expressing neurons in the ventromedial striatum found in the 1.0 mA group is not above the proportion of Arc expressing neurons induced by exploration of the inhibitory avoidance box or by training with the low foot-shock intensity. Significant differences were evident neither among the Cage, 0.0, 0.5 mA, and SO-2 groups nor between the 1.0 and 2.0 mA groups.

Similarly, in the ventrolateral region of the striatum the one-way ANOVA showed significant differences in the proportion of Arc expressing neurons among the groups ($F_{(5,18)}$ = 10.33, $p < 0.0001$); the post hoc Bonferroni test also showed a significant increase in the 2.0 mA group as compared to the Cage, 0.0, 0.5 mA, and SO-2 groups ($p´s < 0.001$, 0.005, 0.004, and 0.001, respectively). The proportion of Arc expressing neurons in the 1.0 mA group was significantly higher with respect to the Cage and SO-2 groups ($p < 0.05$ in both comparisons). Significant differences were evident neither among the Cage, 0.0, 0.5 mA, and SO-2 groups nor between the 1.0 and 2.0 mA groups (Figure 4B).

These results indicate that, as in the case of non-contingent foot-shock and the 0.0 mA groups, training with the lower foot-shock did not induce changes in Arc expressing neurons. On the other hand, moderate training induced an increase in these neurons, which was even greater with intense training both in ventromedial and ventrolateral striatal neurons. Thus, the increased Arc protein expression in ventral-medial and ventral-lateral striatum very probably reflects the recruitment of more neurons by the intense training.

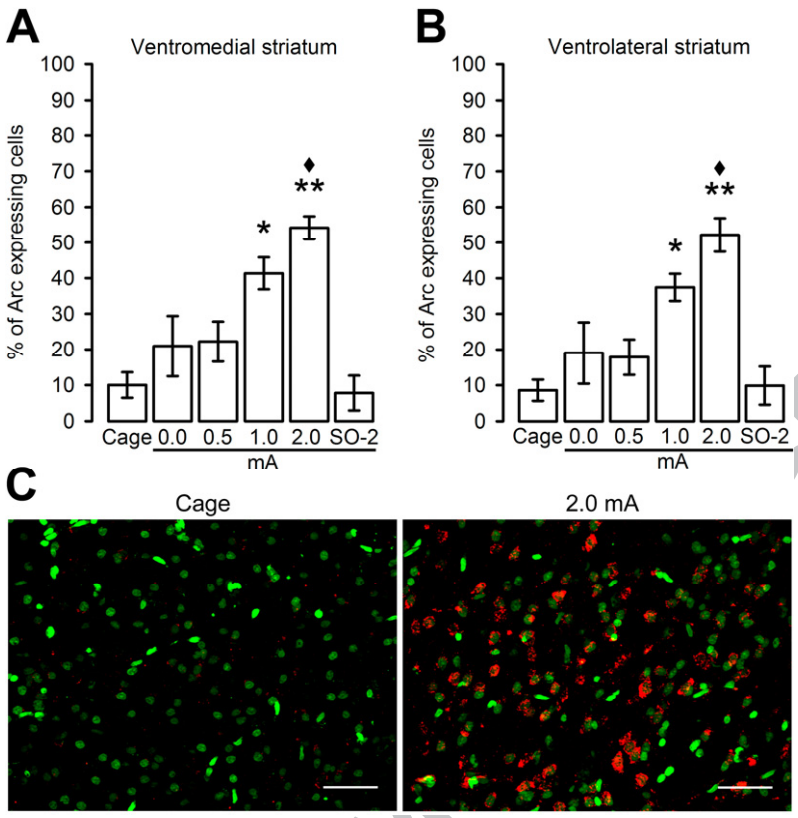

Figure 4. Effects of intensity of inhibitory avoidance training on Arc cell count in ventromedial and ventrolateral striatum. The graphs show the mean (± SEM) percentage of Arc protein expressing cells in the ventromedial striatum (A) and ventrolateral striatum (B). In both regions the 1.0 and the 2.0 mA groups differed significantly from the Cage and SO-2 groups (* $p < 0.05$; ** $p < 0.001$). Additionally, in both regions the 2.0 mA group showed increased Arc expressing neurons compared to the 0.0 and 0.5 mA groups (♦ $p < 0.01$). No significant differences between the 1.0 and 2.0 mA groups were found (n = 4 per group). (C) Representative two dimensional maximum intensity Z projection image from tridimensional z-stack images, labeled for Arc protein (red) and the cell nuclei (green). The left image is from a Cage animal, while the right image is from a 2.0 mA trained animal. Scale bar = 50 µm.

5. Increase in serum corticosterone concentration is related to foot-shock intensity but not to inhibitory avoidance training

As an index of strength of training, we investigated whether CORT serum concentration was related to the intensity of the foot-shocks used for inhibitory avoidance training. De Kloet, Vreugdenhil, Oitzl, & Joëls (1998), reported that CORT release usually reaches maximum levels within 15 min after hypothalamic-pituitary-adrenal (HPA) axis activation. For this reason, groups of rats (n = 4 per group) were trained in inhibitory avoidance, with the same intensities (0.0, 0.5, 1.0, and 2.0 mA) and control conditions (Cage and SO-2 groups) as described above, and then sacrificed 15 min after training; blood was collected at this time for CORT serum concentration measurement. We also evaluated CORT serum concentration in the animals that were sacrificed for Arc expression analysis 45 min after the inhibitory avoidance task (experiment described above; Section 4).

5.1 Radioimmunoassay procedure

The concentration of CORT in serum was determined by radioimmunoassay, using the sheep anti-CORT polyclonal antibody (AB1297, Merck-Millipore) at 1:9000 dilution. Serum samples were diluted 1:500 in 0.01 M phosphate buffer pH 7.5, with 0.15 M NaCl and 0.1% gelatin and heated at 98 °C for 10 min. Linear portion of the standard curve: 5–4,000 pg of CORT/ml (CORT, catalog number 46148, Sigma-Aldrich) (Vetranal analytical standard), samples were assayed in duplicate.

5.2 Results

The one-way ANOVA yielded significant differences among the groups regarding concentration of CORT in serum at 15 min after training ($F_{(5, 13)} = 10.55$, $p < 0.0005$). The post hoc Bonferroni test showed a significantly higher concentration of CORT in the 2.0 mA group than in the Cage and the 0.5 mA groups ($p < 0.05$ for both comparisons); the SO-2 group had a higher amount of CORT than that of the Cage, 0.0, and 0.5 mA groups ($p´s < 0.001$, 0.001, and 0.005, respectively) (Figure 5A). The analysis of CORT concentration 45 min after the training and control procedures did not reveal significant differences among the groups (ANOVA $F_{(5,18)} = 1.48$, $p = 0.24$) (Figure 5B). The high foot-shock clearly increased serum glucocorticoid concentration at the earlier time tested (15 min) which normalized 45 min after training. Thus, serum CORT concentration increased in response to the higher foot-shock intensity, regardless of whether the animals had been trained or not.

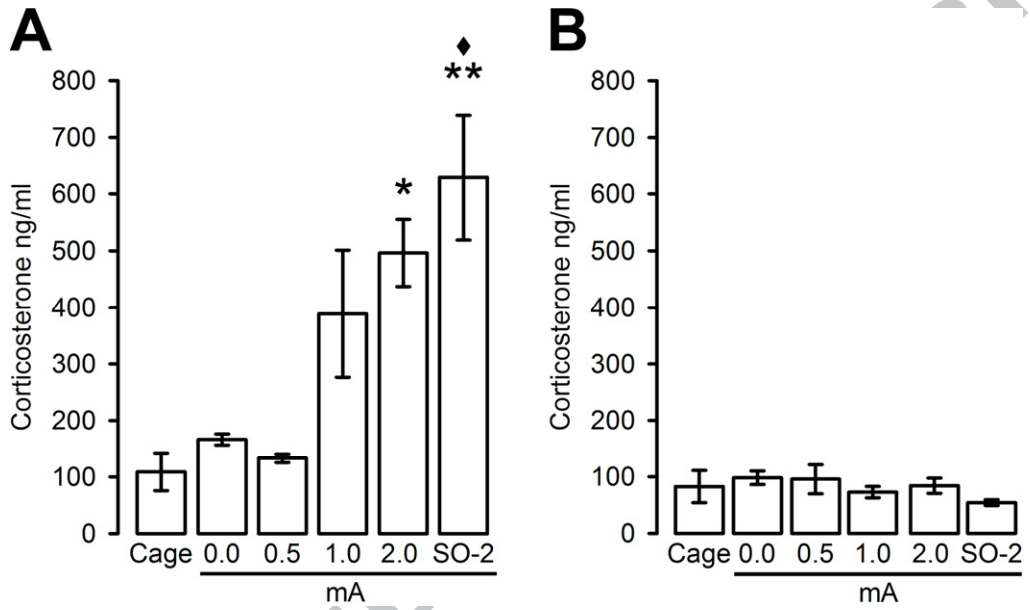

Figure 5. Serum corticosterone concentration is related to high foot-shock intensity. Mean (± SEM) CORT serum concentration, measured at 15 min after training *p < 0.05, **p < 0.005 vs. the Cage and 0.5 mA groups; ♦p < 0.01 vs. the 0.0 mA group (A). No significant differences were found at 45 min after training (B); n = 4 per group.

6. Discussion

The main results of the present study are: 1) training with a high foot-shock intensity produced a higher resistance to extinction than training with moderate or low foot-shock intensities; 2) the group trained with the high foot-shock intensity had a larger area of Arc expression in ventral striatum than the moderate and low foot-shock groups, while no changes in Arc expression were produced in dorsal striatum by any of the treatments; 3) training with both moderate and high foot-shock intensities increased the number of neurons that express Arc protein in ventral striatum, but not in dorsal striatum.

Intense inhibitory avoidance training, using a foot-shock of 2.0 mA, produced a memory trace that was highly resistant to extinction (Figure 1), contrasting with the animals trained with 1.0 mA, which on the first test session showed a similar retention latency to the former group, but on subsequent sessions showed rapid extinction just as the animals trained with the lower foot-shock did. These results confirm previous observations, where training with a foot-shock of relatively high-intensity also induced a higher resistance to extinction of inhibitory avoidance learning (Bello-Medina et al., 2016; Garín-Aguilar et al., 2012; Prado-Alcalá et al., 1994, 2012).

The increase of about 40% in the number of Arc protein expressing cells found in the ventral regions, in both the 1.0 mA and 2.0 mA groups, does not seem to be congruent with the finding that there was a significantly larger Arc signal area in the 2.0 mA group than in the 1.0 mA group. This apparent discrepancy might be due to a wider distribution of Arc protein in the soma and dendrites of striatal neurons of the animals that had been trained with 2.0 mA. However, the methods

used in the present study did not permit us to properly test this possibility because we did not measure Arc protein expression quantitatively, and because the histological procedures used did not allow for an optimal preservation of the dendritic structure. Nonetheless, by measuring Arc protein concentration in dendritic fractions with Western blotting, previous reports showed that administering clenbuterol or CORT into the amygdala immediately after inhibitory avoidance training increased dendritic Arc protein concentration in hippocampus, cingulate, and prefrontal cortex; these treatments also improved inhibitory avoidance performance in a retention test run 48 h after training (McIntyre et al., 2005; McReynolds et al., 2010; McReynolds, Holloway-Erickson, Parmar, & McIntyre, 2014).

The specificity of the effects of training on Arc expression was verified by the results obtained in the Cage group, which was intentionally manipulated like the other groups in order to factor out the expression of Arc in the trained groups. As it was expected, the amount of Arc-expressing cells in the Cage group did not differ from the groups that were only exposed to the inhibitory avoidance box or to the foot-shock alone.

It should be pointed out that spatial exploration is known to induce Arc expression in about 20% of striatal neurons (Vazdarjanova et al., 2006); we also found that about 20% of ventral striatal neurons express Arc after the rats explored the inhibitory avoidance box (0.0 mA group) or after inhibitory avoidance training with the low foot-shock intensity (0.5 mA), suggesting that exploration, by itself, may account for the low amount of neurons expressing Arc in these conditions. The fact that the group trained with the low foot-shock intensity did not show an

increment in Arc-expressing cells points to the idea that a certain threshold in the intensity of the aversive stimulation used for training must be reached to induce a significant increment of Arc.

Interestingly, we found that high CORT levels in blood serum were produced after training with the high foot-shock intensity; however, the increase in CORT release was also found in the group that received the non-contingent high foot-shock. This outcome leaves open the question of whether the inhibitory avoidance training by itself produced the release of CORT, since this result was confounded by the fact that the aversive stimulation, alone or administered during training was accompanied by such release.

A very important fact is that the increase in the expression of Arc protein in the ventral striatum was not induced in the high foot-shock untrained group, indicating that expression of Arc in the ventral striatum is not linked to the aversive stimulation per se, as the CORT release is, but rather to the complex associative processes derived from the interaction among the context, the high foot-shock, and the motor performance involved in IA.

Taken together, this set of findings demonstrates that, compared with low and moderate training, intense inhibitory avoidance training induces Arc protein expression in a higher number of neurons in ventral striatum, and, probably, a higher amount of Arc in these neurons. The role of Arc in long-term synaptic plasticity is well documented (Bramham, Worley, Moore, & Guzowski, 2008; Nonaka et al., 2014; Steward & Worley, 2002). The increased expression of Arc protein produced by intense training could be involved in the generation of robust synaptic modifications, such as those seen in striatal dendritic spine density and

morphology following intense inhibitory avoidance training (Bello-Medina et al., 2016). These synaptic modifications are probably involved in the mechanisms underlying the strong resistance to extinction shown by the animals subjected to intense training. For this reason, we consider that evaluation of Arc expression gives reasonable clues about the neuronal mechanisms involved in enhanced learning.

The two measures used to analyze Arc protein expression, consistently indicated that the ventral regions of the striatum were the only regions where this expression was augmented after intense training. This may be explained by their anatomical and functional features. They communicate with different cortical and limbic regions (Berendse, Graaf, & Groenewegen, 1992; McGeorge & Faull, 1989; Ramanathan, Hanley, Deniau, & Bolam, 2002; Voorn et al., 2004). Importantly, the ventrolateral striatal region communicates with the insular cortex (Lasiter, Deems, & Glanzman, 1985), which is involved in aversively motivated associations (Bermudez-Rattoni, 2014), and it has been proposed that the ventrolateral striatum is involved in integrating the affective attributes of associated stimuli (Lelos, Harrison, & Dunnett, 2011).

In conclusion, training in the inhibitory avoidance task with a foot-shock of moderate intensity produces good retention of the task and an increment of active neurons in ventral, but not in dorsal striatum, as was also the case after training with the high-intensity foot-shock. However, only intense training induced high resistance to extinction and a larger area of Arc expression signal in the ventrolateral region, thus giving support to the hypotheses that guided this work.

This Arc expression may be related to augmented synaptic plasticity, and may be one of the physiological substrates of enhanced learning.

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8. References

Bello-Medina, P. C., Flores, G., Quirarte, G. L., McGaugh, J. L., & Prado Alcalá, R. A. (2016). Mushroom spine dynamics in medium spiny neurons of dorsal striatum associated with memory of moderate and intense training. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(42), E6516–E6525. http://doi.org/10.1073/pnas.1613680113

Berendse, H. W., Graaf, Y. G.-D., & Groenewegen, H. J. (1992). Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *The Journal of Comparative Neurology*, *316*(3), 314–347. http://doi.org/10.1002/cne.903160305

Bermudez-Rattoni, F. (2014). The forgotten insular cortex: Its role on recognition memory formation. *Neurobiology of Learning and Memory*, *109*, 207–216. http://doi.org/10.1016/j.nlm.2014.01.001

Bramham, C. R., Worley, P. F., Moore, M. J., & Guzowski, J. F. (2008). The immediate early gene arc/arg3.1: regulation, mechanisms, and function. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(46), 11760–7. http://doi.org/10.1523/JNEUROSCI.3864-08.2008

Cobos-Zapiaín, G. G., Salado-Castillo, R., Sánchez-Alavez, M., Quirarte, G. L., Roldán-Roldán, G., Díaz del Guante, M. A., & Prado-Alcalá, R. A. (1996). High Level of Footshock during Inhibitory Avoidance Training Prevents Amnesia Induced by Intranigral Injection of GABA Antagonists. *Neurobiology of Learning and Memory*, *65*(3), 202–206. http://doi.org/10.1006/nlme.1996.0024

Cordero, M. I., Merino, J. J., & Sandi, C. (1998). Correlational relationship between

shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. *Behavioral Neuroscience*, *112*(4), 885–891. http://doi.org/10.1037/0735-7044.112.4.885

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*, *19*(3), 269–301. http://doi.org/10.1210/edrv.19.3.0331

Devan, B. D., & White, N. M. (1999). Parallel information processing in the dorsal striatum: relation to hippocampal function. *The Journal of Neuroscience*, *19*(7), 2789–98.

Díaz-Trujillo, A., Contreras, J., Medina, A. C., Silveyra-Leon, G. A., Antaramian, A., Quirarte, G. L., & Prado-Alcalá, R. A. (2009). Enhanced inhibitory avoidance learning prevents the long-term memory-impairing effects of cycloheximide, a protein synthesis inhibitor. *Neurobiology of Learning and Memory*, *91*(3), 310–4. http://doi.org/10.1016/j.nlm.2008.10.006

Dudman, J. T., & Gerfen, C. R. (2015). The Basal Ganglia. In G. Paxinos (Ed.), *The Rat Nervous System* (pp. 391–440). http://doi.org/10.1016/B978-0-12-374245-2.00017-6

Durán-Arévalo, M., Cruz-Morales, S. E., & Prado-Alcalá, R. A. (1990). Is acetylcholine involved in memory consolidation of over-reinforced learning? *Brain Research Bulletin*, *24*(6), 725–727. http://doi.org/10.1016/0361-9230(90)90129-N

Gardner, R. S., Suarez, D. F., Robinson-Burton, N. K., Rudnicky, C. J., Gulati, A., Ascoli, G. A., & Dumas, T. C. (2016). Differential Arc expression in the hippocampus and striatum during the transition from attentive to automatic

navigation on a plus maze. *Neurobiology of Learning and Memory*, *131*, 36–45. http://doi.org/10.1016/j.nlm.2016.03.008

Garín-Aguilar, M. E., Díaz-Cintra, S., Quirarte, G. L., Aguilar-Vázquez, A., Medina, A. C., & Prado-Alcalá, R. A. (2012). Extinction procedure induces pruning of dendritic spines in CA1 hippocampal field depending on strength of training in rats. *Frontiers in Behavioral Neuroscience*, *6*, 12. http://doi.org/10.3389/fnbeh.2012.00012

Garín-Aguilar, M. E., Medina, A. C., Quirarte, G. L., McGaugh, J. L., & Prado-Alcalá, R. A. (2014). Intense aversive training protects memory from the amnestic effects of hippocampal inactivation. *Hippocampus*, *24*(1), 102–112. http://doi.org/10.1002/hipo.22210

Goodman, J., Leong, K.-C., & Packard, M. G. (2012). Emotional modulation of multiple memory systems: implications for the neurobiology of post-traumatic stress disorder. *Reviews in the Neurosciences*, *23*(5–6), 627–43. http://doi.org/10.1515/revneuro-2012-0049

Guzowski, J. F. (2002). Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. *Hippocampus*, *12*(1), 86–104. http://doi.org/10.1002/hipo.10010

Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of Activity-Dependent Arc Protein Expression in the Rat Hippocampus Impairs the Maintenance of Long-Term Potentiation and the Consolidation of Long-Term Memory. *The Journal of Neuroscience*, *20*(11), 3993–4001.

Guzowski, J. F., McNaughton, B. L., Barnes, C. A., & Worley, P. F. (1999).

Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nature Neuroscience*, *2*(12), 1120–1124. http://doi.org/10.1038/16046

Guzowski, J. F., Setlow, B., Wagner, E. K., & McGaugh, J. L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *The Journal of Neuroscience*, *21*(14), 5089–5098. http://doi.org/21/14/5089 [pii]

Hyman, S. E. (2005). Addiction: a disease of learning and memory. *The American Journal of Psychiatry*, *162*(8), 1414–22. http://doi.org/10.1176/appi.ajp.162.8.1414

Kemp, J. M., & Powell, T. P. (1971). The structure of the caudate nucleus of the cat: light and electron microscopy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *262*(845), 383–401. http://doi.org/10.1098/rstb.1983.0080

Kreitzer, A. C., & Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron*, *60*(4), 543–54. http://doi.org/10.1016/j.neuron.2008.11.005

Lasiter, P. S., Deems, D. A., & Glanzman, D. L. (1985). Thalamocortical relations in taste aversion learning: I. Involvement of gustatory thalamocortical projections in taste aversion learning. *Behavioral Neuroscience*. US: American Psychological Association. http://doi.org/10.1037/0735-7044.99.3.454

Lelos, M. J., Harrison, D. J., & Dunnett, S. B. (2011). Impaired sensitivity to Pavlovian stimulus-outcome learning after excitotoxic lesion of the ventrolateral neostriatum. *Behavioural Brain Research*, *225*(2), 522–8. http://doi.org/10.1016/j.bbr.2011.08.017

Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U., & Kuhl, D. (1995). Somatodendritic expression of an immediate early gene is regulated by synaptic activity. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(12), 5734–8.

Lozano, Y. R., Serafín, N., Prado-Alcalá, R. A., Roozendaal, B., & Quirarte, G. L. (2013). Glucocorticoids in the dorsomedial striatum modulate the consolidation of spatial but not procedural memory. *Neurobiology of Learning and Memory*, *101*, 55–64. http://doi.org/10.1016/j.nlm.2013.01.001

Lyford, G. L., Yamagata, K., Kaufmann, W. E., Barnes, C. A., Sanders, L. K., Copeland, N. G., … Worley, P. F. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron*, *14*(2), 433–445. http://doi.org/10.1016/0896-6273(95)90299-6

McGaugh, J. L. (2013). Making lasting memories: Remembering the significant. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(Suppl 2), 10402–10407. http://doi.org/10.1073/pnas.1301209110

McGeorge, a J., & Faull, R. L. M. (1989). The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience*, *29*(3), 503–537. http://doi.org/10.1016/0306-4522(89)90128-0

McIntyre, C. K., Miyashita, T., Setlow, B., Marjon, K. D., Steward, O., Guzowski, J. F., & McGaugh, J. L. (2005). Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of*

America, *102*(30), 10718–23. http://doi.org/10.1073/pnas.0504436102

McReynolds, J. R., Donowho, K., Abdi, A., McGaugh, J. L., Roozendaal, B., & McIntyre, C. K. (2010). Memory-enhancing corticosterone treatment increases amygdala norepinephrine and Arc protein expression in hippocampal synaptic fractions. *Neurobiology of Learning*, *93*(3), 312–21. http://doi.org/10.1016/j.nlm.2009.11.005

McReynolds, J. R., Holloway-Erickson, C. M., Parmar, T. U., & McIntyre, C. K. (2014). Corticosterone-induced enhancement of memory and synaptic Arc protein in the medial prefrontal cortex. *Neurobiology of Learning and Memory*. http://doi.org/10.1016/j.nlm.2014.02.007

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals* (8th ed). Washington DC: National Academy of Sciences. http://doi.org/10.17226/12910

Neill, D. B., & Grossman, S. P. (1970). Behavioral effects of lesions or cholinergic blockade of the dorsal and ventral caudate of rats. *Journal of Comparative and Physiological Psychology*, *71*(2 PART 1), 311–317. http://doi.org/10.1037/h0029110

Nonaka, M., Fujii, H., Kim, R., Kawashima, T., Okuno, H., & Bito, H. (2014). Untangling the two-way signalling route from synapses to the nucleus, and from the nucleus back to the synapses. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1633), 20130150. http://doi.org/10.1098/rstb.2013.0150

Okuno, H. (2011). Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers. *Neuroscience Research*, *69*(3), 175–86.

http://doi.org/10.1016/j.neures.2010.12.007

Packard, M. G., & Knowlton, B. J. (2002). Learning and memory functions of the Basal Ganglia. *Annual Review of Neuroscience*, *25*(1), 563–593. http://doi.org/10.1146/annurev.neuro.25.112701.142937

Parsons, R. G., & Ressler, K. J. (2013). Implications of memory modulation for post-traumatic stress and fear disorders. *Nature Neuroscience*, *16*(2), 146–53. http://doi.org/10.1038/nn.3296

Pérez-Ruiz, C., & Prado-Alcalá, R. A. (1989). Retrograde amnesia induced by lidocaine injection into the striatum: Protective effect of the negative reinforcer. *Brain Research Bulletin*, *22*(4), 599–603. http://doi.org/10.1016/0361-9230(89)90076-2

Prado-Alcalá, R. A. (1995). Serial and Parallel Processing During Memory Consolidation. In J. L. McGaugh, F. Bermúdez-Rattoni, & R. A. Prado-Alcalá (Eds.), *Plasticity in the central nervous system : learning and memory* (pp. 57–65). Mahwah, NJ: L. Erlbaum Associates.

Prado-Alcalá, R. A., & Cobos-Zapiaín, G. G. (1977). Learning deficits induced by cholinergic blockade of the caudate nucleus as a function of experience. *Brain Research*, *138*(1), 190–196. http://doi.org/10.1016/0006-8993(77)90797-1

Prado-Alcalá, R. A., Haiek, M., Rivas, S., Roldan-Roldan, G., & Quirarte, G. L. (1994). Reversal of extinction by scopolamine. *Physiology & Behavior*, *56*(1), 27–30. http://doi.org/10.1016/0031-9384(94)90257-7

Prado-Alcalá, R. A., Medina, A. C., López, N. S., & Quirarte, G. L. (2012). Intense emotional experiences and enhanced training prevent memory loss induced by post-training amnesic treatments administered to the striatum, amygdala,

hippocampus or substantia nigra. *Reviews in the Neurosciences*, *23*(5–6), 501–508. http://doi.org/10.1515/revneuro-2012-0061

Prado-Alcalá, R. A., Salado-Castillo, R., Quiroz, C., Garín-Aguilar, M. E., Díaz-Trujillo, A., Rivas-Arancibia, S., & Quírarte, G. L. (2007). Enhanced learning protects brain against effects of amnesic treatments. In F. Bermúdez-Rattoni (Ed.), *Neural Plasticity and Memory: From Genes to Brain Imaging* (pp. 175–191). Boca Raton (FL): CRC Press. http://doi.org/doi:10.1201/9781420008418.ch9

Prado-Alcalá, R. A., & Wise, R. A. (1984). Brain stimulation reward and dopamine terminal fields. I. Caudate-putamen, nucleus accumbens and amygdala. *Brain Research*, *297*(2), 265–273. http://doi.org/10.1016/0006-8993(84)90567-5

Quiroz, C., Martínez, I., Quirarte, G. L., Morales, T., Díaz-Cintra, S., & Prado-Alcalá, R. A. (2003). Enhanced inhibitory avoidance learning prevents the memory-impairing effects of post-training hippocampal inactivation. *Experimental Brain Research*, *153*(3), 400–2. http://doi.org/10.1007/s00221-003-1704-1

Ramanathan, S., Hanley, J. J., Deniau, J.-M., & Bolam, J. P. (2002). Synaptic convergence of motor and somatosensory cortical afferents onto GABAergic interneurons in the rat striatum. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(18), 8158–8169.

Ramírez-Amaya, V., Vazdarjanova, A., Mikhael, D., Rosi, S., Worley, P. F., & Barnes, C. A. (2005). Spatial exploration-induced Arc mRNA and protein expression: evidence for selective, network-specific reactivation. *The Journal of Neuroscience*, *25*(7), 1761–8. http://doi.org/10.1523/JNEUROSCI.4342-

04.2005

Salado-Castillo, R., Díaz del Guante, M. A., Alvarado, R., Quirarte, G. L., & Prado-Alcalá, R. A. (1996). Effects of Regional GABAergic Blockade of the Striatum on Memory Consolidation. *Neurobiology of Learning and Memory*, *66*(2), 102–108. http://doi.org/10.1006/nlme.1996.0051

Salado-Castillo, R., Sánchez-Alavéz, M., Quirarte, G. L., Martínez García, M. I., & Prado-Alcalá, R. A. (2011). Enhanced training protects memory against amnesia produced by concurrent inactivation of amygdala and striatum, amygdala and substantia nigra, or striatum and substantia nigra. *Frontiers in Behavioral Neuroscience*, *5*, 83. http://doi.org/10.3389/fnbeh.2011.00083

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. http://doi.org/10.1038/nmeth.2089

Schwabe, L., Joëls, M., Roozendaal, B., Wolf, O. T., & Oitzl, M. S. (2012). Stress effects on memory: an update and integration. *Neuroscience and Biobehavioral Reviews*, *36*(7), 1740–9. http://doi.org/10.1016/j.neubiorev.2011.07.002

Solana-Figueroa, R., Salado-Castillo, R., Quirarte, G. L., Galindo, L. E., & Prado-Alcalá, R. A. (2002). Enhanced inhibitory avoidance training protects against the amnesic effect of p-chloroamphetamine. *Life Sciences*, *71*(4), 391–399. http://doi.org/10.1016/S0024-3205(02)01684-3

Steward, O., & Worley, P. (2002). Local synthesis of proteins at synaptic sites on dendrites: role in synaptic plasticity and memory consolidation? *Neurobiology of Learning and Memory*, *78*(3), 508–527.

http://doi.org/10.1006/nlme.2002.4102

Torregrossa, M. M., Corlett, P. R., & Taylor, J. R. (2011). Aberrant learning and memory in addiction. *Neurobiology of Learning and Memory*, *96*(4), 609–23. http://doi.org/10.1016/j.nlm.2011.02.014

Vazdarjanova, A., McNaughton, B. L., Barnes, C. A., Worley, P. F., & Guzowski, J. F. (2002). Experience-Dependent Coincident Expression of the Effector Immediate-Early Genes Arc and Homer 1a in Hippocampal and Neocortical Neuronal Networks. *The Journal of Neuroscience*, *22*(23), 10067–10071.

Vazdarjanova, A., Ramirez-Amaya, V., Insel, N., Plummer, T. K., Rosi, S., Chowdhury, S., … Barnes, C. A. (2006). Spatial exploration induces ARC, a plasticity-related immediate-early gene, only in calcium/calmodulin-dependent protein kinase II-positive principal excitatory and inhibitory neurons of the rat forebrain. *The Journal of Comparative Neurology*, *498*(3), 317–329. http://doi.org/10.1002/cne

Voorn, P., Vanderschuren, L. J. M. J., Groenewegen, H. J., Robbins, T. W., & Pennartz, C. M. A. (2004). Putting a spin on the dorsal-ventral divide of the striatum. *Trends in Neurosciences*, *27*(8), 468–474. http://doi.org/10.1016/j.tins.2004.06.006

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of Learning and Memory*, *77*(2), 125–184. http://doi.org/10.1006/nlme.2001.4008

Yager, L. M., Garcia, A. F., Wunsch, A. M., & Ferguson, S. M. (2015). The ins and outs of the striatum: Role in drug addiction. *Neuroscience*, *301*, 529–541. http://doi.org/10.1016/j.neuroscience.2015.06.033